REMARKS

Claims 52-70 were pending in the application. Claims 52, 56, 59, 63, 67, and 69 have been amended, and claims 53-55 and 64-66 have been cancelled without prejudice herein. Please add new claims 71-77. Upon entry of this Amendment and Response, claims 52, 56-63, and 67-77 will be pending.

Support for new claims 71 and 74 can be found throughout the claims and specification as originally filed, including at least at page 9, lines 11-18, at page 11, lines 28-31, and Figure 3. *No new matter has been added.*

Cancellations and amendments to the claims should in no way be construed as an acquiescence to any of the Examiner's rejections and were done *solely* to expedite the prosecution of the application. Applicant reserves the right to pursue the cancelled claims in this or a separate application(s).

EXAMINER INTERVIEWS

Applicants thank the Examiner for the courtesy of the personal interview that took place on July 10, 2008 between Examiner Yaen, Examiner Helms, Jeff Browning, Werner Meier, Megan Williams, and Cristin Cowles, during which the new claims and the rejections of record were discussed.

Applicants also wish to thank the Examiner for the courtesy of the personal interview that took place on September 16, 2008 between Examiner Yaen, and Applicant's representatives, Megan Williams and Cristin Cowles. During this second interview, the art cited in the Office Action dated August 1, 2008 was discussed.

THE PENDING CLAIMS ARE NOVEL AND UNOBVIOUS OVER THE CITED ART

The claims as amended are directed to compositions comprising glycosylated, active lymphotoxin-β-receptor immunoglobulin (LT-β-R-Ig) fusion proteins and inactive LT-β-R-Ig fusion proteins, wherein no more than ***10%*** or no more than ***6%*** of the LT-β-R-Ig fusion proteins are in the inactive form. Applicants were the first to discover that there were active and inactive forms of LT-β-R-Ig fusion proteins. Applicants characterized these forms of the molecule and set forth a variety of means by which the active and inactive forms could be separated from each other so that one could arrive at a composition comprising reduced levels of the inactive form according to the claims.

As taught in the instant application, one method by which one can reduce the level of the inactive form present in a composition is by culturing cells at a temperature below 37°C. In order to arrive at a composition comprising glycosylated, active lymphotoxin-β-receptor immunoglobulin (LT-β-R-Ig) fusion proteins and inactive LT-β-R-Ig fusion proteins, wherein no more than 10% the LT-β-R-Ig fusion proteins are in the inactive form, one must culture the cells at a temperature of ***less than 30°C***. None of the cited art teaches or suggests culturing mammalian cells expressing LT-β-R-Ig fusion proteins at such a temperature. Nor does it teach or suggest culturing of mammalian cells expressing other, related molecules or, for that matter, even unrelated molecules at such a temperature. The rejections of the Office Action of August 1, 2008 are addressed in more detail below.

CLAIMS 52-70 ARE NOT OBVIOUS UNDER 35 U.S.C. §103(A) OVER DEGLI ESPOSTI *ET AL.* OR ASHKENAZI *ET AL.* IN VIEW OF RENZETTI *ET AL.* (WO 97/41895) AND ETCHEVERRY (WO 96/039488)

The Examiner has rejected claims 52-70 as being unpatentable over Degli-Esposti *et al.* or Ashkenazi *et al.* (WO 98/25967; hereinafter Ashkenazi) in view of Renzetti *et al.* (WO 97/41895) and Etcheverry (WO 96/039488). The Examiner states that it would be "prima facie obvious to those of ordinary skill in the art at the time the invention was made to optimize the production condition of LTβR-fusion proteins as claims to arrive at a product which would have no more than 30% inactive fusion protein in the composition." Applicant respectfully traverses this rejection.

Amended claims

Set forth above, the amended claims presented herein are directed to compositions comprising glycosylated, active LT-β-R-Ig fusion proteins and inactive LT-β-R-Ig fusion proteins, wherein no *more than **10%** of the LT-β-R-Ig fusion proteins are inactive*. In addition, new claims 72-77 are directed to compositions comprising glycosylated, active LT-β-R-Ig fusion proteins and inactive LT-β-R-Ig fusion proteins, *wherein no more than **6%** of the LT-β-R-Ig fusion proteins are inactive*.

Failure to establish prima facie case of obviousness under 35 USC § 103

Applicant submits that the combination of Degli-Esposti or Ashkenazi in view of either Etcheverry or Renzetti fails to establish a *prima facie* case of obviousness in view of the amended and new claims.

The Examiner relies upon Degli-Esposti for teaching LT-β-R-Ig fusion proteins. Degli-Esposti does *not*, however, teach or suggest, a composition comprising no more than either 10% or 6% active LT-β-R-Ig fusion protein. To make up for the deficiencies of Degli-Esposti, the Examiner cites both Renzetti and Etcheverry. The Examiner states Renzetti teaches "that the production of TNFR-Ig fusion proteins can be optimized by adjusting among other things the temperature to ensure optimum protein production." The Examiner further states that Etcheverry teaches "that controlling the glycosylation patterns of the recombinant protein may affect among other things the physical properties of a recombinant protein." Applicant submits that based on the teachings of Degli-Esposti, either alone or in combination with Renzetti and Etcheverry, one of ordinary skill in the art would not arrive at the claimed compositions.

While Applicant disagrees with the Examiner's assertion that one of ordinary skill would be motivated to apply the teachings of Renzetti and Etcheverry to the fusion protein described in Degli-Esposti, in order to expedite an allowance, independent claims 52 and 63 (and claims that depend there from) have been amended to require that the compositions comprise no more that ***10% inactive*** LT-β-R-Ig fusion protein. New claims 72-77 require that the compositions comprise no more that ***6% inactive*** LT-β-R-Ig fusion protein. The teachings of the references are set forth below:

Degli-Esposti

The Examiner suggests that "[t]hose of skill in the art would have been motivated to generate a higher percentage of 'active' protein over inactive protein because the 'active' form is the working form which can be used for the purpose of treating cancer as taught by Degli-Esposti *et al.*" The Examiner further states that one of ordinary skill in the art would "recognize that a purified form of the fusion protein was needed for the treatment of cancer." Applicant respectfully disagrees with the Examiner's interpretation of the teachings of Degli-Esposti.

Degli-Esposti does *not* teach or suggest that LTβR-fusion protein can be used for any therapeutic purpose, let alone to treat cancer as alleged by the Examiner. The LTβR-fusion protein described in Degli-Esposti was used to *generate antibodies to LTβR*, which in turn

were found to have antiproliferative activity *in vitro* on cancer cell lines. Degli-Esposti discloses that the anti-LTβR antibody M12 can inhibit melanoma cell growth (see first and second paragraphs of Discussion section at page 1760). This finding led the authors of Degli-Esposti to suggest "a potential role for LTβR therapy in the clinical management of tumors." In contrast, LTβR-Fc was found to have the *opposite effect of anti-LTβR antibody M12.* As described in the sentence at page 1758-1759, the authors of Degli-Esposti conclude from experiments that "[t]he specificity of the response generated with the LTβR M12 mAb was confirmed by the finding that the response could be blocked using LTβR-Fc." Thus, one of ordinary skill in the art would not be motivated to "purify" or "generate a higher percentage of 'active' protein" based on the teachings of Degli-Esposti because the reference does not support the use of LTβR-Ig fusion proteins for cancer, as suggested by the Examiner.

Moreover, Degli-Esposti teaches that cells expressing LT-β-R-Ig fusion protein are cultured at 37°C. The reference does not teach or suggest that there are any issues with such culturing conditions. In fact, the reference teaches *a single band of LTβR-Ig fusion protein* when analyzed on a gel. In the section of Degli-Esposti entitled "Generation of soluble recombinant LTβR" at page 1757, the authors teach "[p]urity was assessed using SDS-PAGE analysis, which, under reducing conditions, showed a single band at ~ 65 kDa." Thus, based on the disclosure of Degli-Esposti, one of ordinary skill would not be led to believe that there are two forms of LTβR-Ig fusion protein, as the reference teaches a *single band* suggesting a single species of protein.

Renzetti and Etcheverry

The Renzetti and Etcheverry references teach cells expressing TNF receptor fusion protein, not LT-β-R-Ig. The Examiner relies upon Renzetti and Etcheverry for teaching that low temperature culturing is beneficial "to achieve the highest production of recombinant protein possible."

These references are directed to altering the glycosylation patterns of recombinant TNFR-Ig and teach that methods of increasing production of recombinant molecules result in decreased sialiation of the molecules produced. Both references also teach the benefits of increasing sialic acid for therapeutic use, including to increase plasma half life of the protein (see, for example, pages 20 and 22 of Etcheverry). For example, the Summary of the Invention" at page 2, line 37 to page 3, line 2 of Etcheverry states,

The present inventors have discovered that certain mammalian cell culture

> parameters affect cell specific productivity as well as the extent and type of glycosylation of the proteins produced. More particularly, the present inventors have found that certain factors that enhance cell specific productivity have an inverse effect of the sialic acid content of the produced protein. The present inventors have therefore devised various cell culture parameters to enrich particular glycoforms of glycoproteins produced in mammalian cell culture.

Page 8, lines of Etcheverry also teaches "[t]he present inventors have discovered that factors that increase cell specific productivity during the production of a glycoprotein produced by mammalian cell culture have an *inverse effect on sialic acid content* of the glycoprotein produced" (emphasis added).

Although both of these references provide a range of temperatures at which cells could be cultured, the references are silent with respect to why one would wish to culture cells at a reduced temperature. In actuality, Renzetti and Etcheverry each teach that culture conditions that increase productivity can have a *negative* impact on the protein products produced by cultured cells, as sialic acid content of the resulting protein may be reduced. As noted by the Examiner, the inventors then provide a laundry list of possible parameters that may be altered to increase protein production, including, among others, temperature (see page 8, lines 25-29 of Etcheverry). The references do not teach in which direction temperature should be adjusted, or why. Thus, neither of these references provides the motivation to culture cells expressing LT-β-R-Ig fusion proteins at a reduced temperature.

Assuming arguendo that reduced temperature results in increased production, these references actually teach away from culturing cells at a lower temperature to produce a pharmaceutical product as this would decrease sialiation of the recombinant protein. For example, Etcheverry concludes that "adjustment of these factors, alone or in combination, to increase cell specific productivity generates a protein with decreased sialic acid content" (see page 8, lines 29-30). Similarly, Renzetti at page 8, lines 16-32 teaches the benefits of increasing sialic acid content and the observation that increasing cell culture productivity decreases sialic acid content. In contrast to the Examiner's suggestion, both of these references actually identify a problem with increasing cell culture productivity, *i.e.*, decreased sialic acid content, and provide methods for overcoming this problem. Based on the disclosures of Renzetti and Etcheverry, therefore, one of ordinary skill in the art would ***not*** be motivated to decrease cell culture temperature as Renzetti and Etcheverry disclose that such a method has a negative effect. Thus, in contrast to the Examiner's suggestion that one of skill in the art "without recognizing that there are multiple forms of LTβR fusion protein would

attempt to modify these conditions to achieve the highest production of recombinant protein possible," Renzetti and Etcheverry actually teach away from increasing protein production as it may decrease sialic acid content which is desirable. Thus, Renzetti and Etcheverry do not provide the requisite motivation and expectation of success asserted by the Examiner and required for a *prima facie* case of obviousness.

Moreover, culturing mammalian cells at the temperature ranges taught in these references would ***not*** inherently result in a composition comprising no more than either 10% or 6% inactive LT-β-R-Ig fusion protein. Etcheverry and Renzetti teach temperature ranges of 30°C to 37°C can be used (for Etcheverry see page 3, lines 18-19; page 7, lines 23-24; page 10, lines 38-40; and page 19, lines 16-17; and for Renzetti see page 5, lines 33-34; page 14, lines 20-21; and page 16, lines 27-28). Thus, neither Etcheverry nor Renzetti teaches or suggests lowering the culturing temperature below 30°C. By culturing cells under the conditions taught in these references, the claimed compositions would not inherently be achieved. Accordingly, the cited references fail to teach or suggest all of the claim limitations.

In contrast, Applicant teaches in the specification that the percentage of inactive LT-β-R-Ig fusion protein may be decreased by culturing cells below 30°C. For example, Table II of the specification shows that culturing cells expressing LT-β-R-Ig fusion protein below 30°C, e.g., 28°C, results in 6-10% inactive LT-β-R-Ig fusion protein. In addition, Figure 9 of the instant specification shows that the percentage of inactive LT-β-R-Ig fusion protein obtained from cells incubated at less than 30°C is no more than 10%, as required by the amended claims. Thus, even if one of skill in the art were to combine the teachings of Degli-Esposti with those of Renzetti and/or Etcheverry, which they would not be motivated to do for at least the reasons set forth above, those combined teachings fail to teach or suggest compositions that comprise no more than 10% biologically inactive LT-β-R-Ig fusion protein.

Ashkenazi

The Examiner cited Ashkenazi as a primary reference with Degli-Esposti. However, Ashkenazi does not teach or suggest the LTβR-fusion proteins as required by the claims. Instead, the Ashkenazi reference describes *HVEM polypeptides*, including nucleic acids encoding said and chimeric proteins. The reference also discloses culturing cells expressing this molecule at 37°C. Therefore, as the reference fails to teach or suggest the claimed compositions and fails to teach or suggest culturing mammalian cells expressing any

molecule at a reduced temperature, Applicants request clarification as to why this reference is being cited against the pending claims.

THE OBVIOUSNESS REJECTION SHOULD BE WITHDRAWN

In the Office Action, the Examiner states, citing *Atlas Powder Co. v. Ireco Inc,*, that "[t]he discovery of a previously unappreciated property of a prior art composition...does not render the old composition patentably new to the discoverer.' " The Examiner further states that "the claiming of a new use, new function, or unknown property which is inherently present in the prior art does not necessarily make the claim patentable" (citing *In re Best*). Applicant respectfully submits that the case law cited by the Examiner relates to ***novelty, not to obviousness,*** and to whether a prior art reference inherently anticipates the claimed composition.

With respect to anticipation, as set forth above, the only reference which discloses cells expressing the claimed LT-β-R-Ig fusion protein, describes the culture of cells expressing the molecule at 37°C. The compositions described in Degli-Esposti do not inherently have the percentage of inactive LTβR-Ig fusion proteins required by the amended claims, as Degli-Esposti does not teach production of LTβR-Ig fusion proteins using a method that would decrease the percentage of inactive molecules, *e.g.,* low temperature culturing. The Examiner has not cited a reference that teaches all of the inherent limitations of the claims. The claims are novel over the art of record.

With respect to the obviousness rejection, Etcheverry and Renzetti disclose that mammalian cells expressing a different protein can be cultured at a temperature range of 30°C to 37°C can be used (for Etcheverry see page 3, lines 18-19; page 7, lines 23-24; page 10, lines 38-40; and page 19, lines 16-17; and for Renzetti see page 5, lines 33-34; page 14, lines 20-21; and page 16, lines 27-28). Thus, neither Etcheverry nor Renzetti teaches or suggests lowering the culturing temperature below 30°C. Assuming one were motivated to do so, culturing cells under conditions listed in these references, a composition with the inherent properties of the claimed compositions would not be achieved. Accordingly, the cited references fail to teach or suggest all of the claim limitations.

In view of the above, Applicant respectfully submits that amended claims 52, 56-63, and 66-70 (as well as new claims 71-75) are patentable over Degli-Esposti or Ashkenazi in view of Renzetti and Etcheverry.

THE CLAIMS ARE UNOBVIOUS IN VIEW OF THE STATE OF THE ART AT THE TIME THE INVENTION WAS MADE

Applicant wishes to bring to the attention of the Examiner the references cited in the Supplemental IDS (SIDS) of September 12, 2008. None of the cited references in the SIDS relate to LTβR-Ig proteins as claimed. They were cited to provide insight into the state of the art prior to the filing of the instant application. These references support Applicant's position that it was not routine at the time of filing to reduce the temperature at which any recombinant protein was cultured in order to increase production. While some publications indicate that decreasing temperature to a point had beneficial effects for certain proteins in certain cell types, this was not true for all proteins in all cell types.

As with Renzetti and Etcheverry, a number of references (including Weidemann, Bloemkolk, Borth, Jenkins, Kaufmann, and Chuppa) describe lowering the cell culturing temperature below standard temperatures. *Not all of the references, however, teach that lowering temperature below a certain limit leads to an improved outcome*. Kaufmann, which has a publication date of 1999 (the same as the filing date of the instant application), claims to be the first report of "low-temperature-specific changes at a post-translational level in mammalian cells" (see page 581, first column, second paragraph) and suggests that shifting to 30°C may improve productivity (see Figure 4). With respect to temperatures below 30°C, Kaufmann states, "*molecular response of mammalian cells to low temperatures between 27°C and 32°C is not well understood*" (emphasis added; see page 580, second column, second paragraph). In contrast to Kaufmann, Chuppa teaches the advantages of 34°C (see Conclusion at page 338) and states that the effect of temperature is "clearly cell line dependent" (see page 329, first column, second paragraph). Weidemann describes cell culture temperatures in baby hamster kidney (BHK) cells ranging from 30°C to 37°C and concludes that "[t]hese results suggest that the influence of temperature on the process needs to be determined in each case" (see end of first paragraph at page 115). Similarly, Borth teaches that antibody production at 30°C was not increased in comparison to the standard 37°C (see Figure 1B). Bloemkolk concludes that 37°C is the optimal production and culturing temperature for IgG2a antibodies (see abstract). Thus, as evidenced by the inconsistent teachings of these references, one cannot definitively state that there was a common, general practice of reducing temperature in mammalian cell culture to improve productivity. Even assuming arguendo that such a teaching could be gleaned from these

references, there is certainly no teaching that culturing below 30°C is uniformly advantageous.

Several other references in the supplemental IDS, *i.e.,* the Giard, Ludwig, Rasmussen, and Sureshkumar references, describe modifying temperatures below 30°C. However, the references collectively do not support the notion that it was standard in the art to do so, as some describe negative effects of doing so. For example, Giard teaches temperature shifts from 37°C to either 34°C, 30°C, or 25°C. As described in Table I of Giard, the authors conclude that 34°C and 30°C were optimal temperatures for protein production and that the lower 25°C produced a lower yield. Thus, Giard teaches against reducing the culturing temperature below 34°C or 30°C. Similarly, the Sureshkumar reference describes antibody production at a number of temperatures, including 42°C, 39°C, 37°C, 35°C, 33°C, and 29°C, and concludes that 29°C has a negative effect on cell growth (see Figure 1 of the reference). As such, the authors of the Sureshkumar reference did not even test 29° for protein production (see Figure 2). The Ludwig reference tests a number of temperatures to see the effect on shear stress sensitivity, not protein production, including 39°C, 37°C, 33°C, 30°C, and 28°C. With respect to 28°C, the Ludwig reference states that 28°C has "extremely negative influence on cell growth" (see page 324, first column, first paragraph) but suggests that it may be optimal for resistance to shear stress (something that can occur under particular culture conditions involving sparging). Finally, the Rasmussen PCT application teaches that the most preferred temperature for producing Factor VIII (FVIII) protein in mammalian cells is 27°C. The Rasmussen PCT application does not teach or suggest using this temperature for any other proteins other than Factor VIII. As taught in that application, it was surprisingly found that by culturing mammalian cells at a temperature below 37°C, the yields of ***truncated*** FVIII variants and of FVIII derived subunits (especially FVIII heavy chain) are ***increased*** drastically, both in serum containing and in serum free medium (see page 2, lines 5-10). Truncated variants of LTβR fusion proteins are not produced at low temperature and, moreover, the production of truncated variants of LTβR is not desirable. Accordingly, the disclosure of the Rasmussen reference appears to be limited to the protein taught therein.

In sum, Applicant provided eleven references relating to temperature modifications that may be used in mammalian cell culture. ***Of the eleven references, more than half do not teach culturing mammalian cells below 30°C.*** This finding is in line with the teachings of Renzetti and Etcheverry, the only references that teach culture of a protein related to LTβR and that, as described above, actually teach away from culture conditions that will increase

productivity and lower sialic acid content, of which reduced temperature may be one. Of the references that do test mammalian cell culture conditions below 30°C, about half of these references teach ***against lowering the temperature below 30°C***. Only one reference of the eleven cited describes a benefit to culturing cells below 30°C, *i.e.*, the Rasmussen reference. As noted above, this reference does not suggest extrapolating this culturing condition to any other protein other than Factor VIII. The reference teaches that truncated forms of the protein can be produced at low temperature, an effect that is not desirable for most other proteins, including LTβR.

CONCLUSION

In view of the foregoing comments, reconsideration of the rejections and allowance of all pending claims is respectfully requested.

If a telephone conversation with Applicant's Attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicant's Attorney at (617) 227-7400.

Respectfully submitted,

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